



# Distribution and Characteristics of Microplastics in Barnacles and Wild Bivalves on the Coast of the Yellow Sea, China

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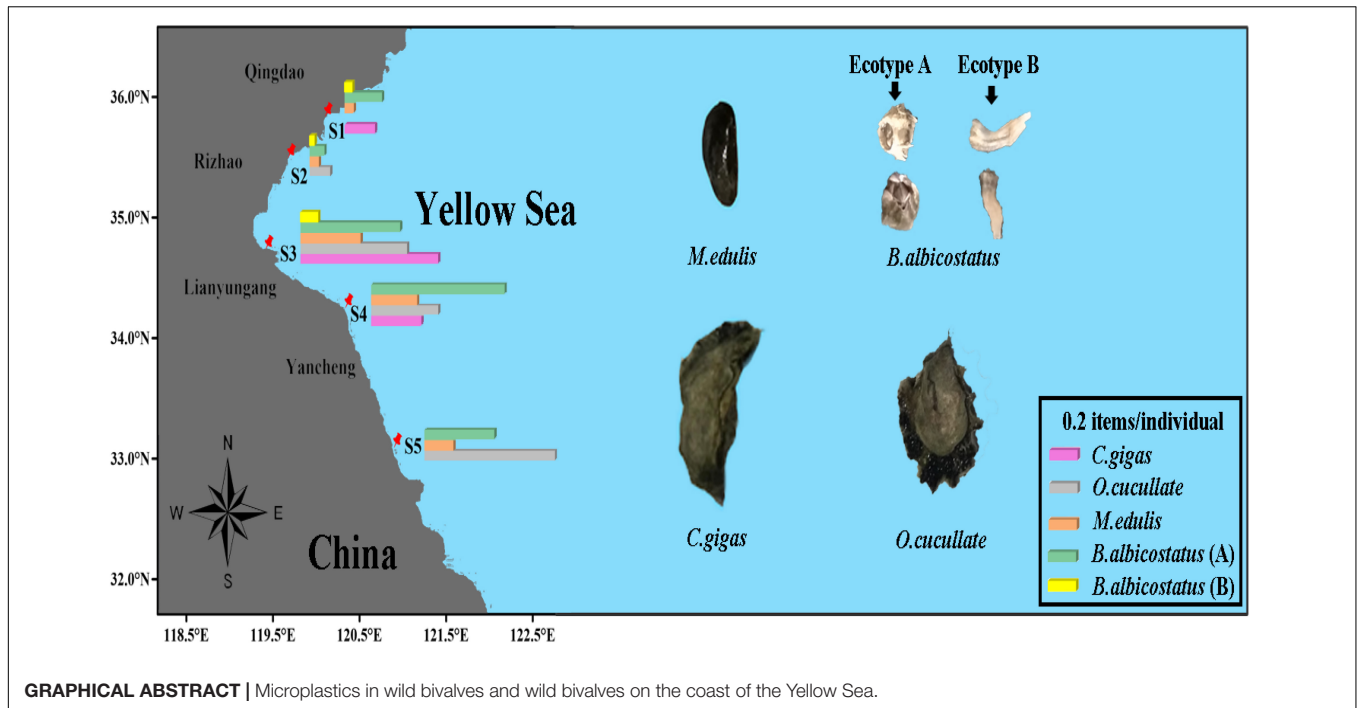
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Barnacles and bivalves are two well-known sessile invertebrates that play important roles in marine ecosystems. Microplastic (MP) pollution has attracted widespread attention. Barnacles and wild bivalves are smaller than farmed individuals; thus, they may be more sensitive to MPs. However, less is known about the abundance and spatial distribution of MPs in wild bivalves along with the coastal areas of China. This study evaluates MP pollution in the most abundant bivalves and barnacles (*Crassostrea gigas*, *Ostrea cucullata*, *Mytilus edulis*, and *Balanus albicostatus*) at five stations in the intertidal zone of the Yellow Sea. *B. albicostatus* was divided into ecotype A and ecotype B. The abundance of MPs in barnacles, wild bivalves barnacles, and wild bivalves varied from 0 to 2.25 items/individual and 0 to 118.21 items/g. *O. cucullata* and *B. albicostatus* (ecotype A) had the highest abundance of MPs, with average abundances of  $0.56 \pm 0.36$  items/individual and  $21.59 \pm 27.26$  items/g, respectively. The types of MPs found in bivalves and barnacles include fibers, fragments, films, and microbeads. The most abundant size was less than 1,000  $\mu\text{m}$ , which accounted for 53% of the total MPs. Cellophane (CP), polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET) were the main polymer types in bivalves and barnacles. This study suggests that the abundance of MPs in wild bivalves is close to that of farmed bivalves with commercial specifications, despite their smaller size. The MP abundance of barnacles in the Yellow Sea is higher than that in other areas in terms of items per gram. In addition, the ecological type may affect the ability of barnacles to accumulate MPs. Ingestion of MPs by barnacles and wild bivalves should be of concern because they may enter the human body through the food web and may pose a potential threat to human health.

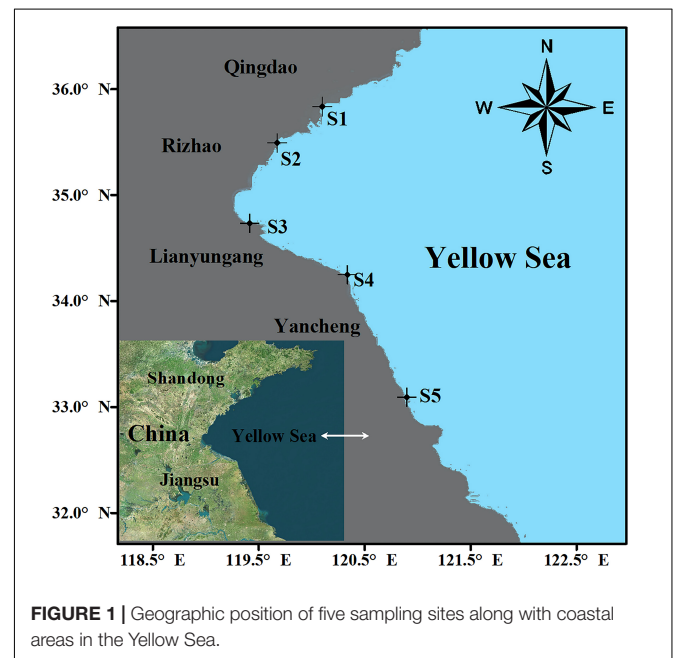
**Keywords:** microplastic, native oysters, mussels and barnacles, pollution, ecosystem



## INTRODUCTION

Since plastic was invented in the middle of the last century, it has changed our life due to its multiple advantages, resulting in the massive replacement of traditional materials with plastic (Thompson et al., 2004; Barnes et al., 2009). Actually, the annual production of plastic in the world exceeded an estimate of 335 million tons in 2016 (Plastics Europe, 2018). A considerable amount of plastic waste is discharged into the ocean due to ineffective disposal by humans. As a result, plastic materials account for 60–80% of all marine debris in the ocean (Gregory and Ryan, 1997). It has become a potentially important menace to the marine environment on this planet (Zalasiewicz et al., 2016).

Microplastics (MPs) are defined as any plastic particles smaller than 5 mm in diameter (Thompson et al., 2004), including primary MPs and secondary MPs. Primary MPs are usually derived from industrial production (Costa et al., 2010), while secondary MPs are produced from mesoplastics and macroplastics by physical damage, photodegradation, oxidation, and biodegradation (Thompson et al., 2004; Auta et al., 2017; Isobe et al., 2019). Due to the small size of MPs, they are more easily dispersed in the ocean than large plastics. Consequently, MPs are ubiquitous in ocean environmental media (Rachel, 2018; Zhao et al., 2018; Zhu et al., 2018; Deanna and Mona, 2019). This leads to the ingestion of MPs by many marine organisms, including plankton (Lima et al., 2014), marine worms (Besseling et al., 2013), sea cucumber (Mohsen et al., 2019), bivalves (Von Moos et al., 2012; Li et al., 2018; Qu et al., 2018; Teng et al., 2019), crab (Zhang et al., 2021), fish (Foekema et al., 2013; Feng et al., 2019; Su et al., 2019), and even sea birds (Provencher et al., 2018). Moreover, MPs have the ability to carry out more toxic matters due to their large specific surface area and are more difficult to

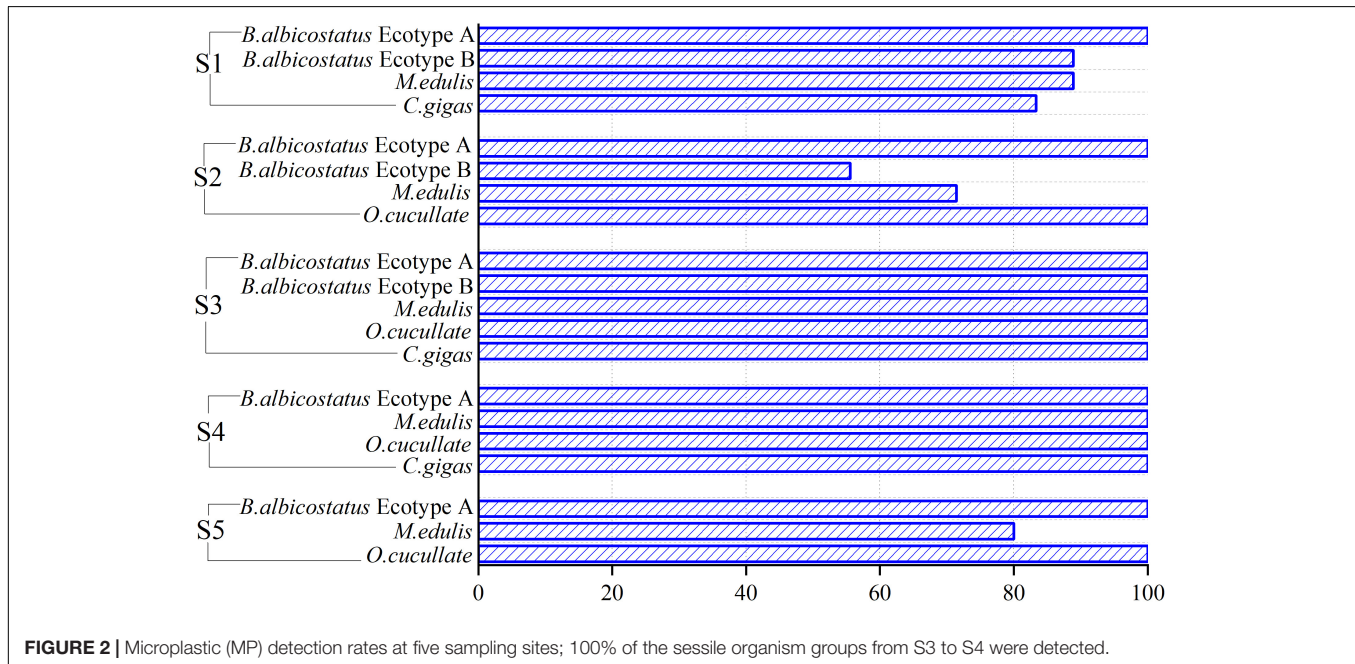


remove (Zhu et al., 2019). Therefore, MPs not only pose a threat to wildlife but are also potentially harmful to the global marine environment (Lebreton et al., 2017; Haward, 2018).

Seafood provides approximately 20% of the animal protein intake of nearly 3 billion people worldwide, and the annual consumption of mollusks per person in China is 9.98 kg (FAO, 2017). During bivalve and barnacle feeding, a large amount of seawater is filtered, consequently accumulating MPs from the seawater. Moreover, previous studies have shown that MPs are

**TABLE 1** | Sampling station information summary.

Sampling sites	Longitude (°E)	Latitude (°N)	Sample weight (g)	Sample number	MPs number
S1	120.05182	35.86453	0.01–2.94	814	71
S2	119.62457	35.52201	0.01–1.49	990	67
S3	119.36516	34.7613	0.01–1.69	755	259
S4	120.28931	34.27538	0.01–2.38	354	151
S5	120.85261	33.11599	0.09–3.17	154	52
Total				3067	600



present in cultured bivalves around the world (Lisbeth and Colin, 2014; Dannielle, 2016; Li et al., 2018; Qu et al., 2018; Teng et al., 2019; Feng et al., 2020c). However, there are few studies on MP pollution in wild bivalves, especially those attached to rocks in the intertidal zone as easily accessible seafood. In addition, bivalves and barnacles play a vital role in coastal ecosystems as an important part of the food chain. Some barnacles and wild bivalves are even thought to be ideal biological indicators in monitoring MP pollution (Li et al., 2018; Qu et al., 2018; Xu et al., 2020). Therefore, it is necessary to conduct more investigations on MP pollution in bivalves and barnacles. Moreover, wild individuals are often small, while cultured individuals are large, which may affect their MP levels. Breeding individuals often use breeding appliances, such as net cages, which are considered to be a source of marine MPs (Feng et al., 2020b). In addition, the difference in density per unit area of wild and cultured individuals is also an important factor affecting the abundance of biological MPs (Sfriso et al., 2020).

This study aims to increase our knowledge about the distribution of MPs in different barnacles and wild bivalves and to quantify MP variability between four species widely distributed in the Yellow Sea, China. The abundance and characteristics of MPs ingested by barnacles and wild bivalves collected from intertidal reefs along the Yellow Sea were investigated. In addition, two

different ecotypes of barnacles were of particular concern. This study provides evidence for future evaluation of the selection of indicator organisms and the ecological and health risks caused by MP pollution of wild seafood in the marine environment.

## MATERIALS AND METHODS

### Sampling Collection

Wild bivalve and barnacle samples were collected at five locations (S1–S5) in the intertidal zone of the Yellow Sea in November 2018 using new stainless steel tools and knives (Figure 1). The collected barnacles and wild bivalves were held in aluminum foil bags. After the collection was completed, they were temporarily stored in a cooler (−5°C) and then stored in a refrigerator at −20°C in the laboratory for further analysis. Prior to dissection and digestion, all wild bivalve and barnacle samples were kept for not more than 3 weeks. *Balanus albicostatus* specimens were divided into two ecotypes based on whether there was a tube at their base (ecotype A without tube and ecotype B with tube).

### Quality Assurance and Control

New tools made of stainless steel were used to collect wild bivalve and barnacle samples. When sampling, researchers in

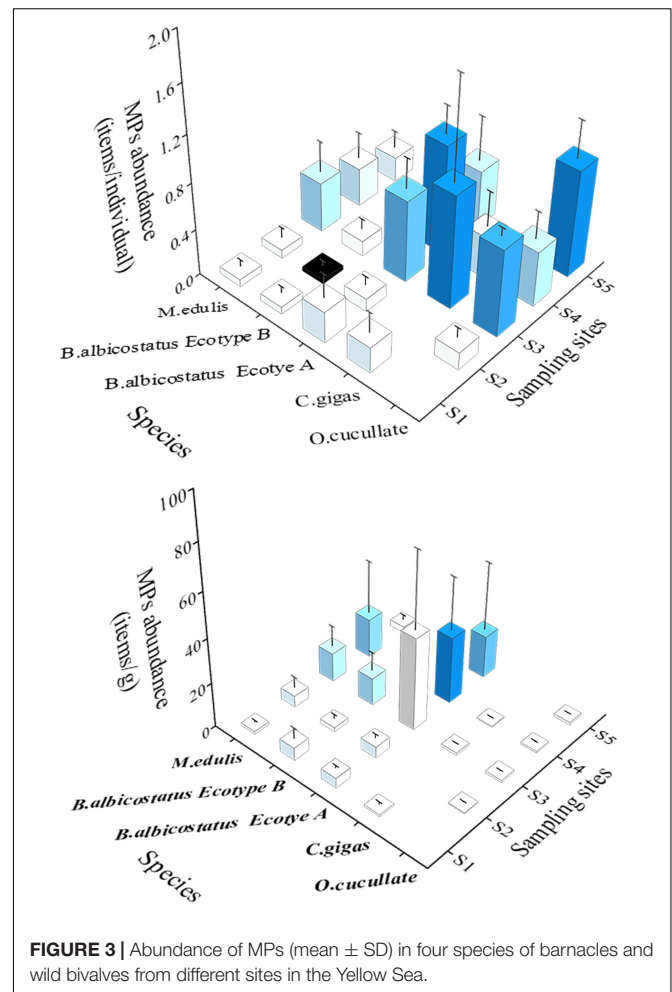
**TABLE 2** | MPs ingestion reported in the literature for sessile invertebrates.

Species	Location	Digestion method	Quantity	References
Mussels	Coast of China	30% H <sub>2</sub> O <sub>2</sub>	2.2 items/g	Li et al., 2016
Mussels	East China Sea	30% H <sub>2</sub> O <sub>2</sub>	9.2 items/g	Kolandhasamy et al., 2018
Mussels	Coast of China	30% H <sub>2</sub> O <sub>2</sub>	1.52–5.36 items/g	Qu et al., 2018
Oysters	Pearl River Estuary, China	10% KOH	1.5–7.2 items/g 1.4–7.0 items/individual	Li et al., 2018
Oysters	Coast of China	10% KOH, 30% H <sub>2</sub> O <sub>2</sub>	0.11–2.35 items/g	Teng et al., 2019
Oysters	the upper Gulf, Thailand	69% HNO <sub>3</sub>	0.37 ± 0.03– 0.57 ± 0.22 items/g	Gajahin et al., 2017
Barnacles	the upper Gulf, Thailand	69% HNO <sub>3</sub>	0.23 ± 0.10– 0.43 ± 0.33 items/g	Gajahin et al., 2017
Barnacles	Coast of Hong Kong, China	10% KOH	0–8.63 items/g 0–1.90 items/individual	Xu et al., 2020
Oysters	The Yellow Sea, China	10% KOH	0.44–1.31 items/g A = 0.87 items/g 0.07–0.85 items/individual A = 0.57 items/individual	This study
Mussels	The Yellow Sea, China	10% KOH	1.20–17.31 items/g A = 7.95 items/g 0.07–0.43 items/individual A = 0.21 items/individual	This study
Barnacles	The Yellow Sea, China	10% KOH	1.88–41.50 items/g A = 17.09 items/g 0.04–0.95 items/individual A = 0.42 items/individual	This study

this study used aluminum foil bags and quickly packed the collected stemless invertebrates separately to reduce atmospheric pollution. Laboratory processing was performed in a laminar flow cabinet (SW-CJ-2F, SUJING, China). Operators in the laboratory were kept to a minimum, and outdoor air circulation was minimized. All liquids were filtered with glass microfibers ( $\Phi = 8 \mu\text{m}$ ,  $d = 47 \text{ mm}$ ) before use. Clothing and instrument cleaning procedures of the operator were consistent with those in our previous studies (Feng et al., 2020b). The MP pollution status in the laboratory passed the program blank (without wild bivalve and barnacle samples) detection, and five program blanks were set for each batch of sample processing.

### Sampling Dissection and Digestion

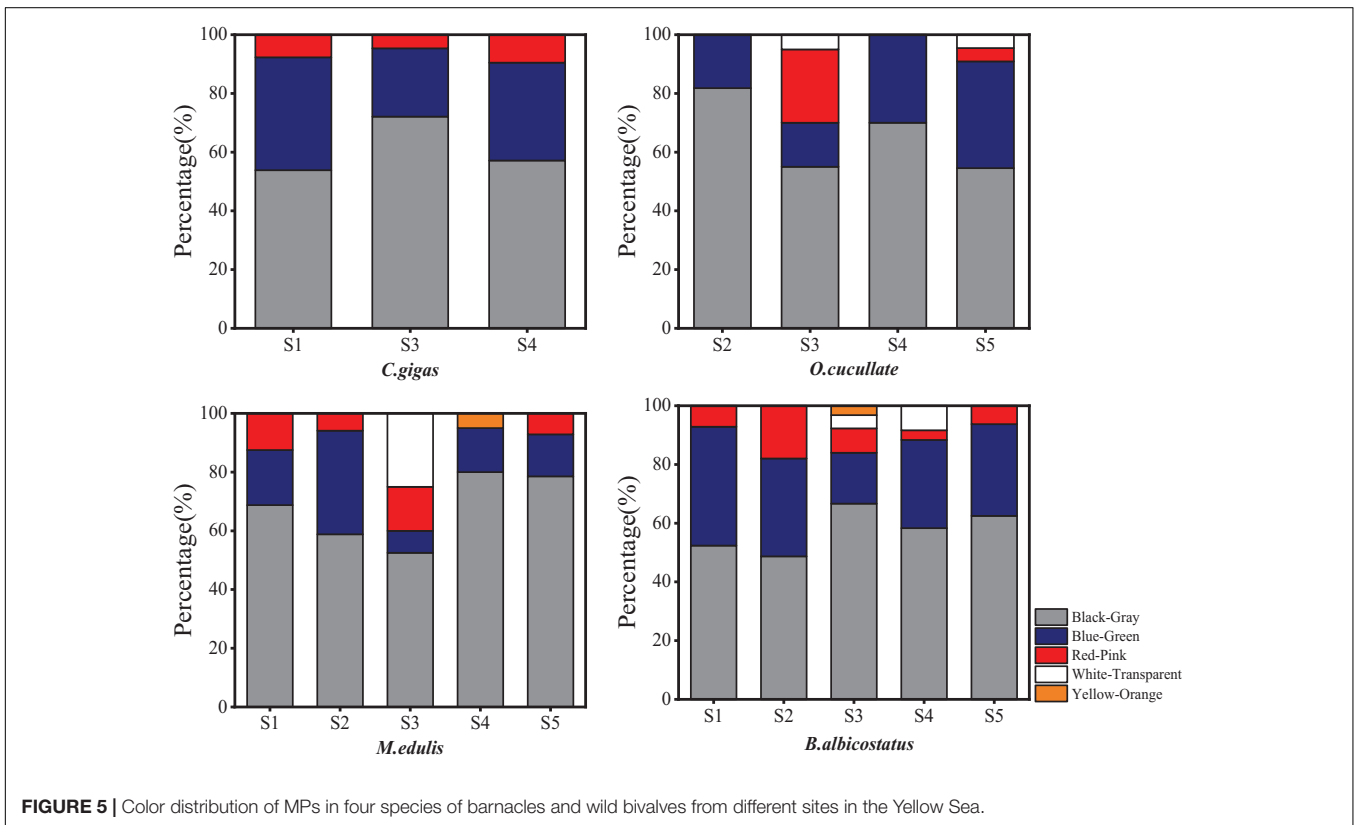
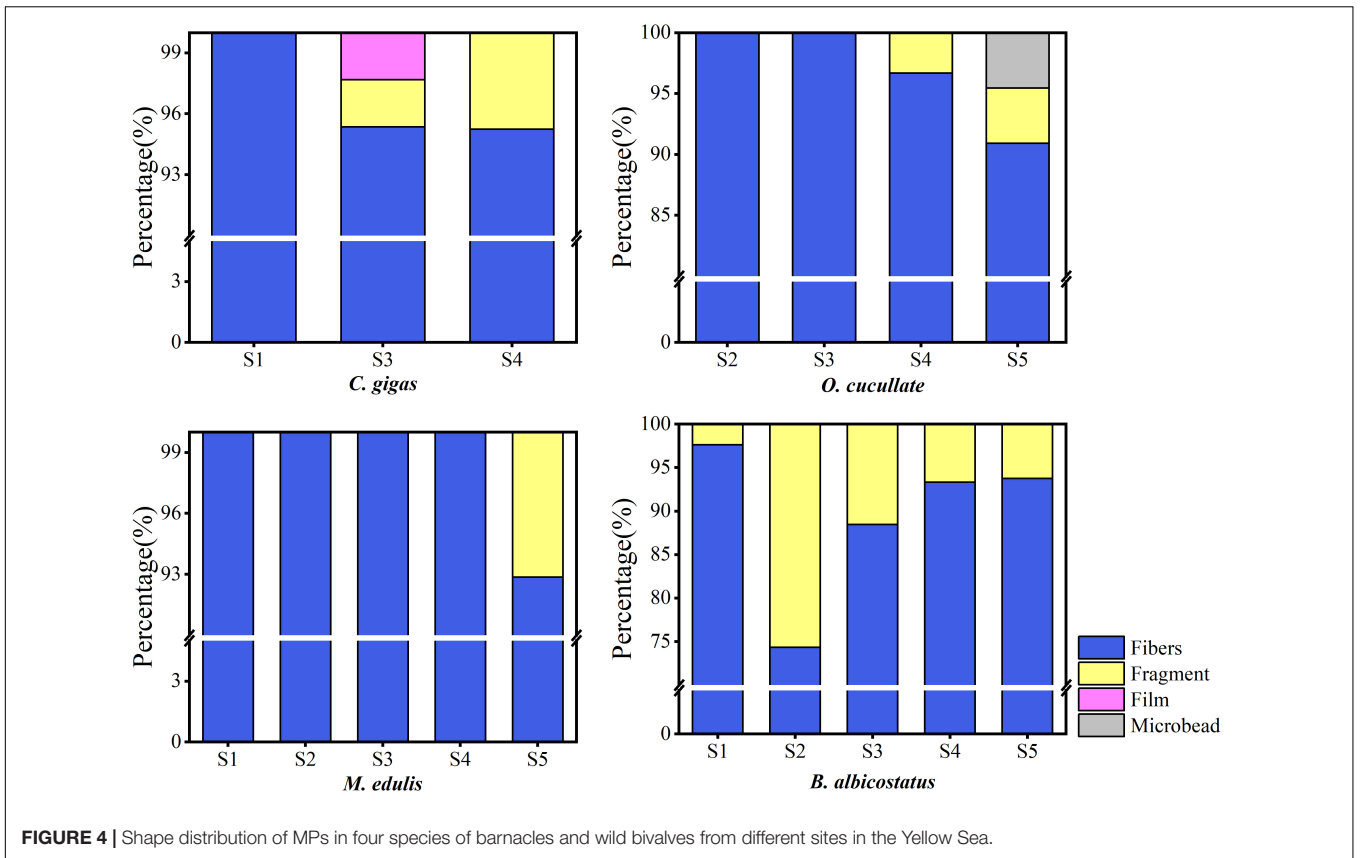
Samples were selected to remove damaged individuals before digestion. The shells of stemless invertebrates were thoroughly rinsed with deionized water after defrosting. Due to their small size and large numbers, the digestion of barnacles and wild

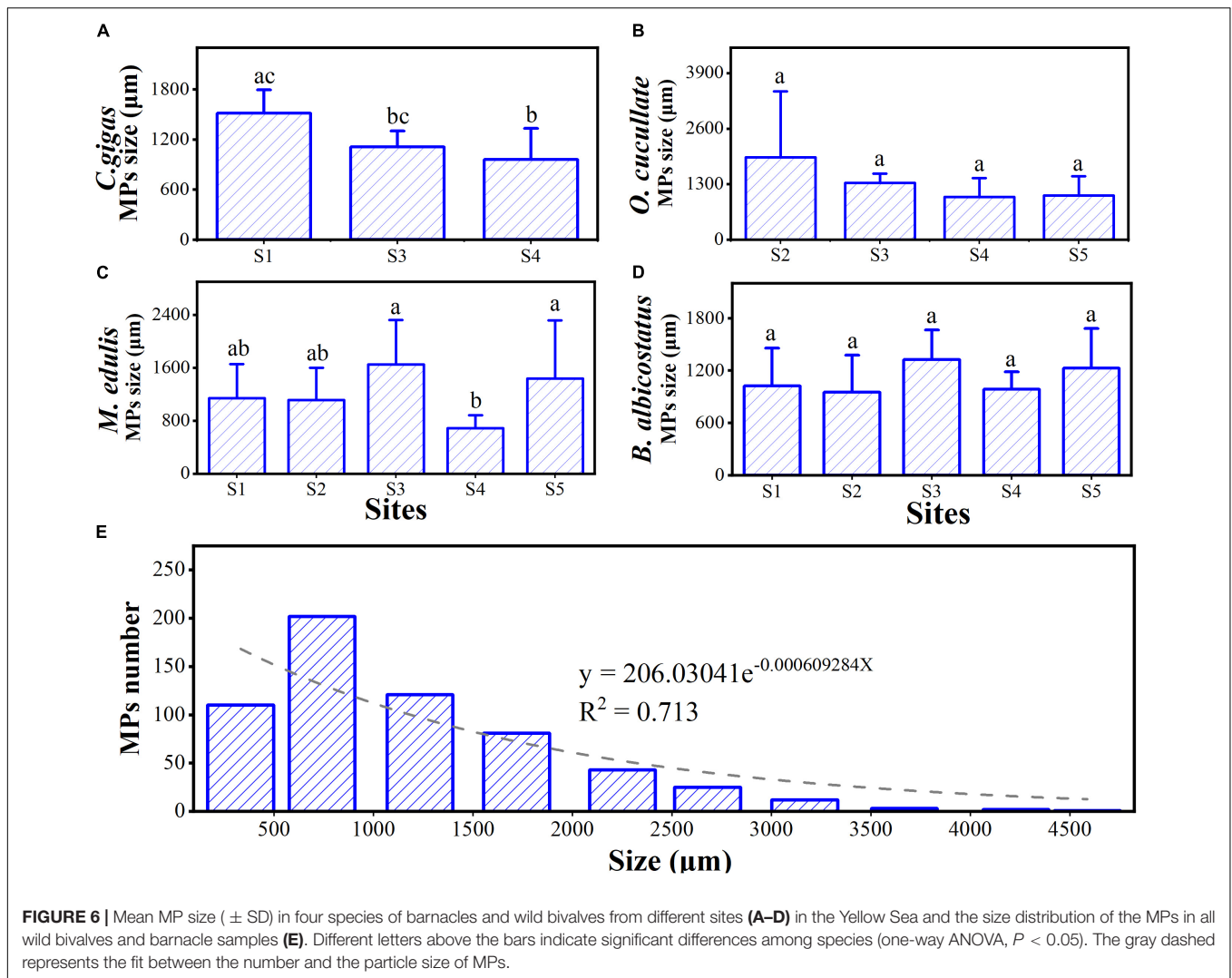


bivalves was not suitable for single sample digestion from the perspective of economy and operability. Therefore, this study uses 30–50 sessile organisms to be digested and processed in groups. The wet weight of soft tissues and total individuals was determined by a precision electronic balance (BS124S, Sartorius Electronic Balance, Beijing). The digestion process was based on previous studies (Foekema et al., 2013; Karami et al., 2017; Feng et al., 2020a). After complete digestion, the clear yellow light digestion solution was transferred and filtered through 8- $\mu\text{m}$  pore size glass microfiber filter membranes (Haining Jinzheng, China) using a filtration unit with one Büchner funnel (AP-01P, Autoscience, Tianjin). The filter membranes were placed in clean Petri dishes with lids and dried at room temperature.

### Microplastic Identification and Data Analysis

The observation and identification of MPs were consistent with previous studies (Zhao et al., 2016; Cai et al., 2019; Feng et al., 2019; Zhang et al., 2021). The data were analyzed using SPSS version 23 (IBM, New York, NY, United States) and visualized using Origin 2021 (Originlab, Northampton, Massachusetts, United States). The MP size data conformed to a normal distribution (Shapiro–Wilk,  $P > 0.05$ ), and the





variances could be considered equal (Levene's test,  $P > 0.05$ ). One-way ANOVA was conducted to assess differences in MP size among different stations. The least significant difference was determined by ANOVA *post hoc* investigation. Kruskal–Wallis ANOVA was used to determine whether there was a significant difference in MP abundance for different species and various sampling stations. A CI of 95% was set for all tests. A linear regression analysis was applied to determine the significant correlation between MP abundance and wild bivalve and barnacle soft tissue weight.

## RESULTS

### Characteristics of Barnacles and Wild Bivalves

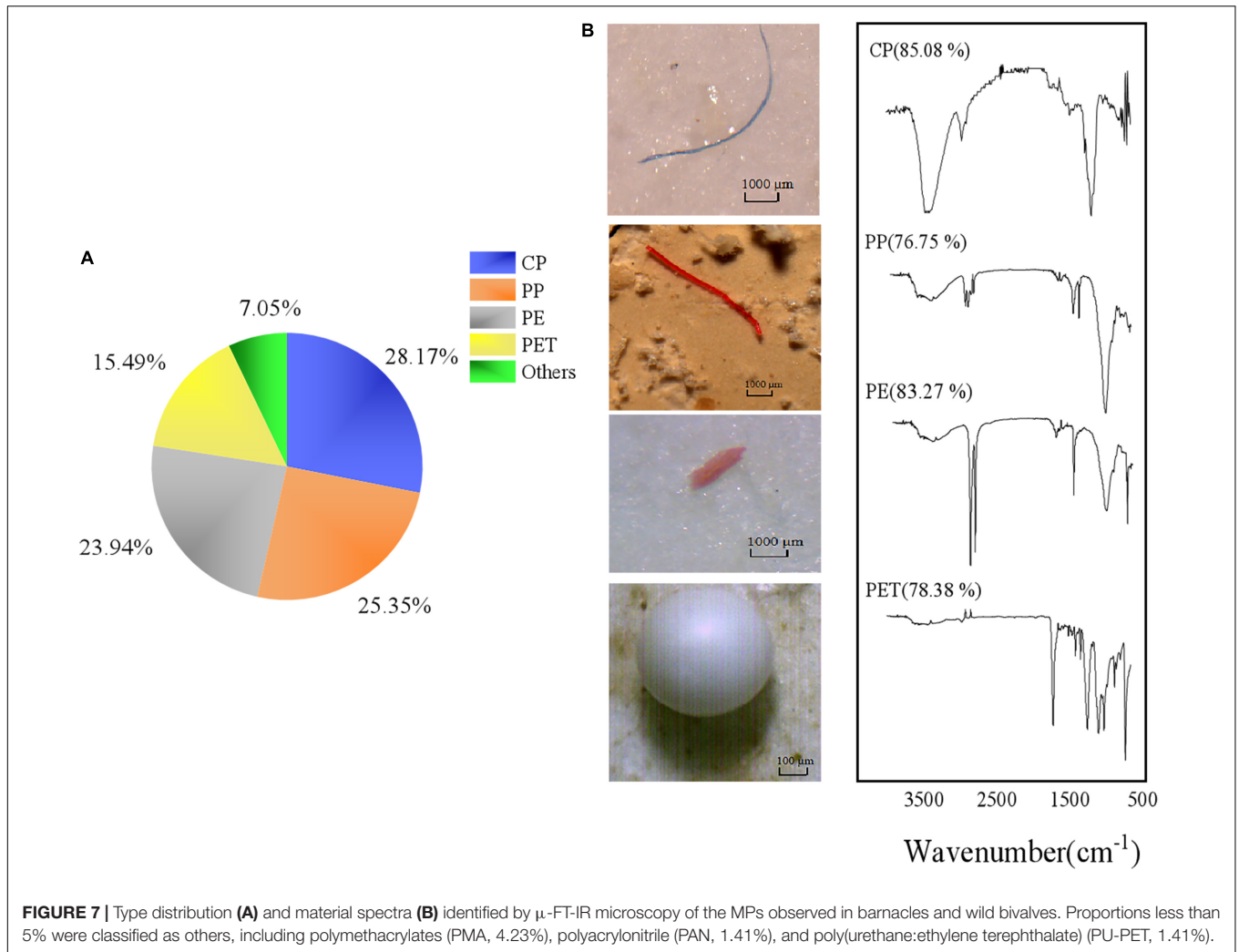
A total of 3,067 barnacles and wild bivalves were collected from five sites along the Yellow Sea (Table 1). These barnacles and wild bivalves were identified as four species, namely, *Crassostrea gigas* (*C. gigas*), *Ostrea cucullata* (*O. cucullata*), *Mytilus edulis*

(*M. edulis*), and *B. albicostatus*. *B. albicostatus* was found in two different ecotypes, one of which had bases without tubes (ecotype A), while the other had bases with tubes (ecotype B), as shown in the Graphical Abstract. The numbers of *C. gigas*, *O. cucullata*, *M. edulis*, *B. albicostatus* (ecotype A), and *B. albicostatus* (ecotype B) were 200, 233, 940, 660, and 1,034, respectively.

### Microplastic Abundance in Barnacles and Wild Bivalves in Different Areas

Microplastics were detected visually in more than 91% of the groups, with a total number of 600 items among all barnacles and wild bivalves. MPs were found in all barnacles and wild bivalves at sites S3 and S4. The lowest detection rate was from S2, which also reached 77.78% (Figure 2).

The MP abundance in barnacles and wild bivalves from five areas was revealed (Figure 3). MP abundance in barnacles and wild bivalves from different areas was significantly different for items per individual (Kruskal–Wallis,  $P = 0.000$ ) and items per gram (Kruskal–Wallis,  $P = 0.000$ ). When both normalized to individual or mass, barnacles and wild bivalves from S3 had



the highest MP abundance ( $0.52 \pm 0.45$  items/individual and  $16.85 \pm 23.42$  items/g), while in terms of items per individual, MP abundance in S2 samples had the lowest level, at  $0.08 \pm 0.08$  items/individual and  $3.13 \pm 3.50$  items/g.

The abundance of MPs of barnacles and wild bivalves for different species was further studied (Figure 3). Kruskal–Wallis ANOVA showed that the MP abundance of various barnacles and wild bivalves was significantly different for both items per individual ( $P = 0.002$ ) and items per gram ( $P = 0.000$ ). The highest MP abundance in items per individual was *O. cucullata* ( $0.56 \pm 0.36$ ), while *M. edulis* was the lowest ( $0.21 \pm 0.21$ ). *B. albicostatus* had the highest value ( $14.09 \pm 21.31$ ), and *C. gigas* was  $0.77 \pm 0.81$  when it was normalized to items per gram.

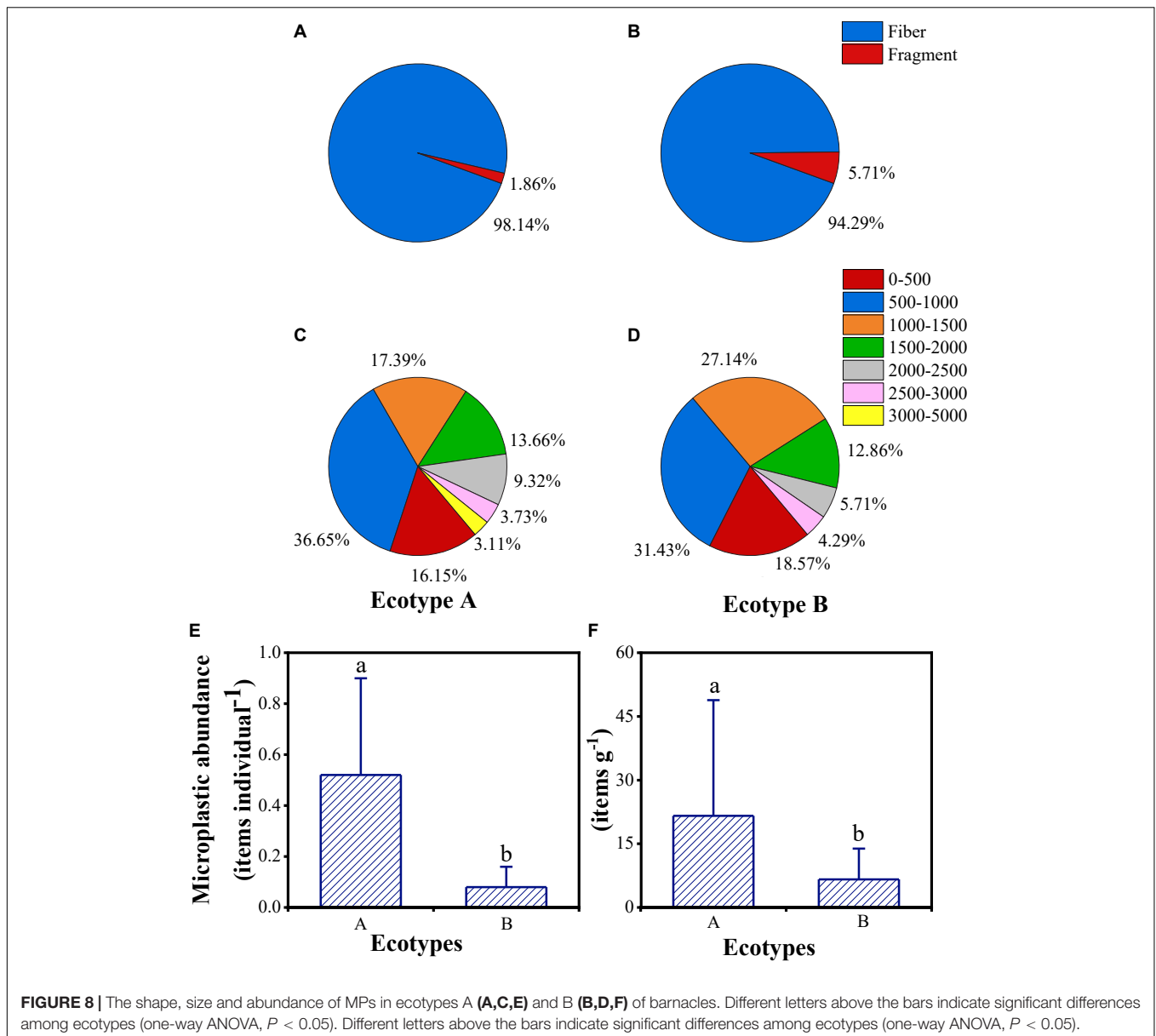
### Characteristics of Microplastics in Barnacles and Wild Bivalves

Multiple types of MPs, including fibers, fragments, films, and microbeads, occurred in the tissues of the barnacles and wild bivalves (Figure 4). The most diverse types were observed as fibers, followed by fragments. The most popular colors were

black, blue, green, and red, which were over 90% (Figure 5). The size of MPs varied from 135.12 to 2,917.69  $\mu\text{m}$ , 86.55 to 4,867.23  $\mu\text{m}$ , 137.85 to 3,331.06  $\mu\text{m}$ , 69.83 to 3,743.20  $\mu\text{m}$ , and 194.66 to 2,885.65  $\mu\text{m}$  in *C. gigas*, *O. cucullata*, *M. edulis*, and *B. albicostatus* ecotype A and ecotype B, respectively. MPs less than 1 mm in size were the most common. As the size of MPs increased, the abundance trend of MPs decreased exponentially ( $R^2 = 0.713$ ,  $P < 0.01$ ) (Figure 6). The materials of suspected MPs in the barnacles and wild bivalves were also confirmed (Figure 7). Most of them were cellophane (CP, 28.17%), followed by polypropylene (PP, 25.35%) and polyethylene (PE, 23.94%).

### Microplastic Pollution Features of Various Ecotypes

The abundance of MPs of different ecotypes and habitats of *B. albicostatus* was also analyzed (Figure 8). Ecotype A barnacles had a higher MP pollution level than ecotype B in terms of both items per individual and items per gram. An average of  $0.52 \pm 0.38$  MPs was found in ecotype A, while  $0.08 \pm 0.08$  MPs were found in ecotype B. In terms of items per gram,



**FIGURE 8 |** The shape, size and abundance of MPs in ecotypes A (A,C,E) and B (B,D,F) of barnacles. Different letters above the bars indicate significant differences among ecotypes (one-way ANOVA,  $P < 0.05$ ). Different letters above the bars indicate significant differences among ecotypes (one-way ANOVA,  $P < 0.05$ ).

$21.59 \pm 27.26$  existed in ecotype A compared with  $6.60 \pm 7.25$  in ecotype B. Both fibers and fragments were found in different *B. albicostatus* ecotypes, but slight differences in proportion existed (fibers occupied 98.14% in ecotype A and 94.29% in ecotype B). There was no significant difference in the particle size distribution of MPs below 3,000  $\mu\text{m}$ . However, MPs with a particle size larger than 3,000  $\mu\text{m}$  were only found in ecotype A barnacles.

### Relationship Between Microplastic Abundance and Soft Tissue Weight of Barnacles and Wild Bivalves

To further explore the relationship between the biological characteristics of sessile organisms and MP pollution levels, the relationship between MP abundance and soft tissue weight was

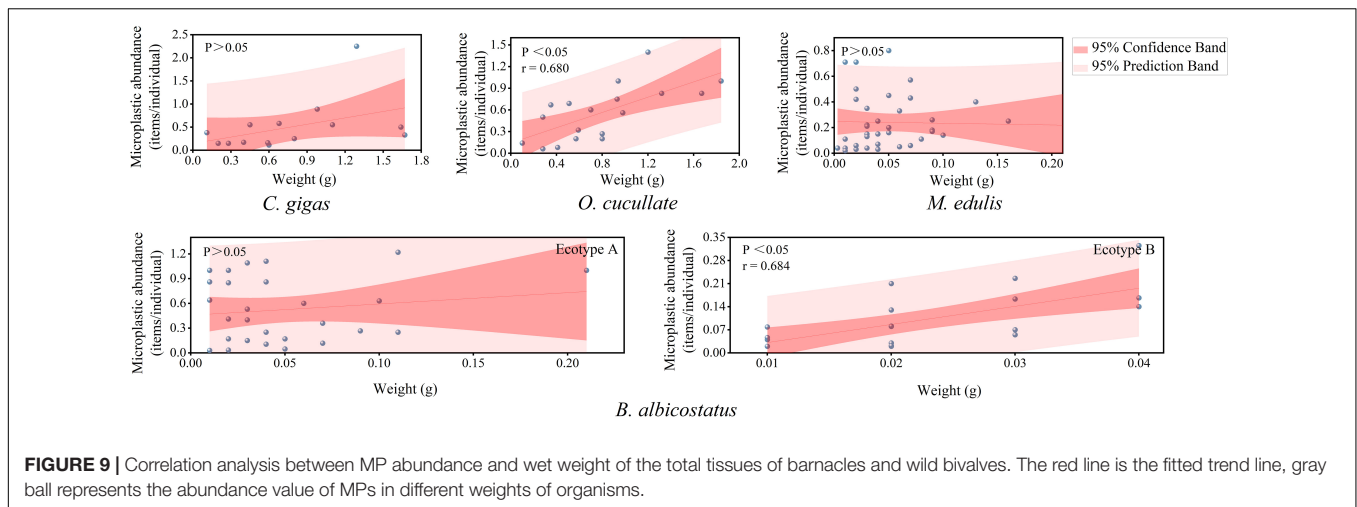
analyzed (Figure 9). The results suggested that the MP value was highly positively related to the tissue weight of *O. cucullata* ( $r = 0.680$ ,  $P < 0.01$ ) and *B. albicostatus* ecotype B ( $r = 0.684$ ,  $P < 0.01$ ) normalized to items per individual.

## DISCUSSION

### Microplastic Pollution Level in Barnacles and Wild Bivalves of the Yellow Sea

In this study, MP abundance in four species of barnacles and wild bivalves in the Yellow Sea was investigated. The high MP detection rate (91.85%) indicates that MPs are widespread in barnacles and wild bivalves, suggesting that barnacles and wild bivalves can accumulate MPs. These results indicate that the





intertidal zone of the Yellow Sea has been contaminated by MPs (Teng et al., 2019; Feng et al., 2020c). Therefore, barnacles and wild bivalves can be exposed to MPs in natural environments. However, the abundance of MPs in the barnacles and wild bivalves was significantly different, indicating significant spatial variability (Figure 8). The highest abundance of MPs and 100% MP detection rate were found in the barnacles and wild bivalves from the S3 region, whether calculated by an individual or by items per gram. The results were also consistent with previous studies on MPs in marine invertebrates (Carney and Eggert, 2019; Teng et al., 2019; Feng et al., 2020a,b), showing that the S3 area is more seriously affected by human activities, such as shipping and mariculture.

In contrast, the abundance of MPs showed great variation between the species of barnacles and wild bivalves, with range values of 0–2.25 items/individual and 0–118.21 items/g. The ability to ingest or eliminate MPs may lead to interspecific differences in MP abundance (Xu et al., 2020). Compared with previous studies (Table 2), the values of MP abundance in mussels showed a higher level by items per gram (Li et al., 2016; Qu et al., 2018), which was only lower than the results of Kolandhasamy et al. (2018). However, it was much lower when expressed by individuals (Li et al., 2016; Kolandhasamy et al., 2018; Qu et al., 2018). For the oysters in this study, both items per gram and items per individual showed intermediate levels of contamination compared with traditional studies (Gajahin et al., 2017; Li et al., 2018; Teng et al., 2019). Few studies have been conducted on MPs in barnacles, and our results show that the pollution level of barnacles in the Yellow Sea is much higher than that in the upper gulf in Thailand and the coast of Hong Kong in terms of items per gram (Gajahin et al., 2017; Xu et al., 2020). When calculated for individuals, the abundance of MPs in barnacles along the coast of Hong Kong was higher than that in this study (Xu et al., 2020). According to the current research results, the uptake of MPs by barnacles and wild bivalves is ubiquitous, and the degree of habitat pollution and the size of individuals are the main influencing factors. In particular, MPs are found in oysters and mussels, although they are not cultured on ropes or cages. In addition, it is worth noting that

due to different methods, there may be differences in comparing the levels and abundance of MP pollution between different studies (Table 2).

## Characteristics of Microplastic Pollution in Barnacles and Wild Bivalves

In this study, MPs with a size of <1,000  $\mu\text{m}$  were the most abundant, which has also been found in many previous studies (Peng et al., 2017; Teng et al., 2019; Feng et al., 2020a). Browne et al. (2011) reported that MPs with small particle sizes may be more likely to be ingested and to accumulate in tissues, and MPs with small particle sizes may show extremely high toxic effects on copepod adults and offspring. In terms of the shape of particles in barnacles and wild bivalves, most MPs were fibrous, which was consistent with the results from seawater samples from the Yellow Sea (Sun et al., 2018). In the South Yellow Sea, the high-frequency use of plastic fishing tools (such as fishing gear, ropes, and fishing nets) has led to fibers becoming the most common shape in this area (Feng et al., 2019). In addition, fibers may also be the most abundant shape of MPs in the ocean (Frias et al., 2016; Andrés and Ruth, 2017). For materials, CP, PP, PE, and polyethylene terephthalate (PET) were the main polymer types in the barnacles and wild bivalves. These four material types are also the most abundant materials for MPs in coastal environments and organisms (Jabeen et al., 2017; Zhu et al., 2018; Feng et al., 2019; Mohsen et al., 2019; Teng et al., 2019; Zhang et al., 2019).

## Microplastic Pollution in Different Ecotypes of Barnacles

In our study, the distribution of barnacles was found at Stations S1–S5. Among them, two types of barnacles with different ecotypes (ecotype A without tubes and ecotype B with tubes) were found at Stations S1–S3. The abundance of MPs in these two different ecotypes of barnacles was different, and the abundance of MPs in barnacles with tubes was significantly lower than that of tubeless barnacles. In addition, the characteristics of MPs of different ecotypes of barnacles are manifested only in the significant differences in the abundance of MPs, and there was no significant difference in type. These results may indicate

that differences in ecotypes affect the accumulation of MPs in marine organisms (Figure 8). In this study, during the sampling process, we found that barnacles with ecotype B had a higher population density than those with ecotype A. We speculate that when the environment has a certain number of MPs, the larger the number of individuals is, the smaller the number of MPs that can be obtained. There were similar inferences in a study of MP pollution of the benthos (Sfriso et al., 2020). However, the mechanism of MP accumulation in individuals caused by different ecological types still needs further confirmation. In previous studies, the abundance of MPs in two different ecotypes of *Ulva prolifera* (drifting and attached) showed significant differences, which provides evidence supporting our conclusion (Feng et al., 2020b). We speculate that ecotypes may play an important role in the accumulation of MPs, not only in plants but also in animals. Thus, we suggested that the selection of MP indicator organisms should consider in-depth their ecological type (Figure 9).

### Characteristics of Microplastic Pollution in Barnacles and Wild Bivalves

A large number of studies have confirmed that MPs can cause damage to marine invertebrates, including mechanical damage caused by abrasion or obstruction of the digestive tract, reduction in a filter-feeding rate and growth rate, energy consumption, and oxidative stress (Von et al., 2012; Rochman et al., 2013; Wright et al., 2013). In addition, the large specific surface area of MPs allows MPs to interact with pollutants in the environment. In particular, MPs have a strong adsorption capacity for persistent organic pollutants (POPs, Rochman et al., 2013; Wright et al., 2013). This adsorption behavior allows MPs to act as carriers for contaminants to enter the organism through the food chain (Derraik, 2002; Cole et al., 2011). In addition, barnacles and wild bivalves play an important ecological function in the intertidal zone. They are not only important feeders of phytoplankton and zooplankton but also serve as prey for various marine animals. This bioaccumulation effect of MPs may gradually have a negative impact on the diversity of coastal organisms and the functionality of ecosystems.

Mussels, oysters, and some species of barnacles are important sources of seafood along the coast of China. Barnacles and wild bivalves distributed in the intertidal zone are also easy to obtain for consumption. Furthermore, they are consumed whole, without gut removal. As a result, the consumption of this contaminated seafood may increase the potential risk to human health (Hussain et al., 2001). However, detailed investigations should be conducted to understand the mutual transmission and accumulation of MPs through the food webs of coastal and

marine ecosystems and the harmful effects for marine life in regard to MP ingestion.

## CONCLUSION

Our study found that MP pollution in barnacles and wild bivalves is widespread in the intertidal zone of the Yellow Sea. Barnacles and wild bivalves can be used as ecological indicators of MPs. Different ecotypes may lead to differences in the accumulation of MPs in barnacles. However, the mechanism of MP accumulation in different ecotypes of barnacles remains to be studied. In addition, our study indicates that MPs are widespread in seafood, such as shellfish, representing a potential risk to human health.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## AUTHOR CONTRIBUTIONS

TZ: conceptualization, methodology, writing original draft – reviewing and editing, supervision, and funding acquisition. KS: investigation, methodology, formal analysis, and visualization. LM: conceptualization, methodology, and writing, reviewing, and editing. RT and TS: investigation. WH: methodology. ZF: conceptualization, formal analysis, writing original draft, reviewing and editing, supervision, and funding acquisition. All authors contributed to the article and approved the submitted version.

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